

The University of Chicago

THE STRUCTURAL BASIS FOR THE
RESPONSE OF THE COMB OF THE
BROWN LEGHORN FOWL TO THE
SEX HORMONES

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
MARY HARDESTY

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Reprinted from
THE AMERICAN JOURNAL OF ANATOMY
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TWO TEXT FIGURES AND FOUR PLATES (THIRTEEN FIGURES)

CONTENTS

Introduction	278
A. Review of work on response	278
B. Statement of principal problems	280
C. Material	280
Histological basis of response	281
A. Structure of mature cock's comb	281
1. Epidermis	281
2. Dermis	282
B. Structure of capon's comb	286
C. The response to the male hormone	288
1. The histological results of injection	288
2. The mechanism of response	289
D. Structure of mature hen's comb	292
E. The response to the female hormone	294
The comb as an organ	297
A. Origin and topography	297
B. External structure of the adult comb	298
C. Embryonic development	299
1. Epidermis	300
2. Dermis	300
D. Development from hatching to maturity	301
1. Gross studies	302
2. Histological studies—differentiation from the common embry- onic type	305
a. Male	306
b. Female	307

¹ This investigation was made possible by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Dr. F. R. Lillie. The author wishes to thank Doctor Lillie for suggesting the problem and for his helpful criticism during the course of the work.

E. Comb transplantations	308
1. Review of previous work	308
2. The unity of stump and graft	309
3. Histological studies of grafts	311
4. Castration and injection in grafted birds	311
Summary and conclusions	313
Literature cited	314

INTRODUCTION

In the sex-hormone work, both in this laboratory and elsewhere, the comb of fowls has been used as a trustworthy and measurable indicator of the presence of the male hormone. The fluctuations of the comb as a result of castration and subsequent introduction of the testis hormone, whether by testicular grafts or by injection of hormone extracts, have been studied by means of the external appearance and gross measurements of the comb. So far, however, no analysis has been made of the histological changes involved. The investigations reported in this paper center around the histology and mechanism of the comb reaction. As a basis for this study, the structure, both gross and microscopic, and the development of the normal male and female combs are considered, in comparison with the comb of the capon.

A. Review of work on response

1. *Regression after castration.* One of the most conspicuous results of prepuberal castration in the male is the failure of the comb and other head furnishings to develop. If castration is performed after puberty, the head furnishings begin to regress immediately after castration, and continue until they reach the typical capon condition. Measurements of the rate of regression have been reported by a considerable number of workers, among whom are Goodale ('16), Pézard ('18), Zawadowsky ('22), Finlay ('25), and Benoit ('29).

2. *Maintenance and restoration by grafting.* Berthold, in 1849, was able to prevent the regression of comb and wattles in castrated cockerels by autoplasmic and homoplasmic transplantation of testis tissue into the abdominal cavity. The fully regressed combs of capons can also be brought back to

the normal cock condition by the establishment of testis grafts. Pézard ('20), Caridroit ('26), Finlay ('25), and Appel ('29) all report the resumption of male secondary sex characters in capons with successful testicular grafts.

3. *Restoration by injection.* Attempts to restore male characters in capons by injection of testis extracts have been numerous. Various methods have been used for the extraction of the hormone from the testis. (For literature, see McGee, Juhn, and Domm, '28.) McGee, working under Dr. F. C. Koch, was the first ('27) to report consistent results. They prepared an extract from bull testes which uniformly produced growth of comb and wattles in capons. The results of this and subsequent work are reported in greater detail in the paper of McGee, Juhn, and Domm ('28). In 1929, Koch and Gallagher were able to obtain a much more highly purified preparation (Moore, Gallagher, and Koch, '29).

In all the work reported above the changes in the comb, whether in growth or regression, have been studied simply by gross measurements of length and height, with the addition, in most cases, of calculations of comb area. None of the workers has investigated the microscopic structure of the comb in the effort to determine of what structures it is composed and how they react.

Champy and Kritch ('26) are the only authors who have attempted any histological analysis of the changes which are evident grossly and who have presented a theory as to the mechanism by which these changes are accomplished. By a Bresiline technique they have succeeded in staining differentially what they call the muco-elastic layer of the cock's comb. After applying the same technique to capon combs, they state that this layer disappears entirely in the capon, and hence conclude that the muco-elastic layer is a direct result of secretion of the male hormone. If the muco-elastic layer is homologous with the region which we have found it convenient to call the intermediate layer, and undoubtedly it is, we cannot agree that this layer disappears entirely after castration, as we shall show.

B. Statement of principal problems

The situation thus outlined makes it desirable to possess a much more complete knowledge of this interesting organ than is now available. In the effort to acquire an understanding of the mechanism by which the comb responds to hormone stimulation, a systematic microscopic as well as macroscopic study has been made of its development from its first appearance in the embryo to the adult male or female state. From this work the gradual differentiation of comb tissue, under the influence of the hormone secreted by the gonad of a sexually maturing bird, becomes apparent. With the structure of the mature cock's comb as a basis for comparison, studies have been made upon the histological changes which take place in the regression of the comb after castration, and its restoration after injection of the male hormone. On the female side, comparisons of the histological features of the combs of mature hens at the time of laying and in the periods between laying have also been made.

Approaching the problem of response from another point of view, autoplasmic comb grafts have been made, and the effect of castration and injection upon both the grafts and the stumps of comb tissue remaining after amputation have been studied. The very striking lack of regenerative power in the comb as an organ has also been considered.

C. Material

The birds used for these experiments came from the stock of Light Brown Leghorns used in this laboratory. The material for the study of comb development after hatching was derived from the 'normal series' collected by Dr. Mary Juhn. I wish here to thank her for permitting me to use it and the data which she collected at the periodic autopsies. Thanks are also due Dr. L. V. Domm for his kindness in performing the necessary castration operations.

The methods by which the studies were made will be indicated as each of the various phases of the work is presented.

HISTOLOGICAL BASIS OF RESPONSE

The material for the histological studies was fixed in Bouin's fluid and sectioned by the celloidin-paraffin method. Sections were stained in Mallory's triple connective-tissue stain, Heidenhain's azan (azo-carmin and aniline blue), and in iron haematoxylin. Ferguson's ('11) modification of the Bielschowsky silver-impregnation method was also applied to comb tissue.

The histological comparisons of mucoid content were made from portions of male, female, capon, and injected-capon combs fixed in ten parts of Zenker's stock to one part of neutral formalin and stained in toluidine blue. The procedure used is, with slight modifications, that being developed by Dr. Sylvia Holton Bensley, of the Hull Anatomical Laboratory.

The extractions of mucoid were made after the method of Chittenden and Gies ('96). The details of this method are described in the presentation of these experiments.

Before considering the reaction of comb tissue to the hormone, a study of the general structural plan of the comb is in order. Since the mature male comb represents the summit of differentiation, we shall consider it first, and follow with the points wherein the comb of the capon differs. Thereafter we shall present a comparison of the histological structure of the female's comb at the time of laying and in between those periods.

A. Structure of the mature cock's comb (pls. 1, 2, and 3)

The comb is morphologically an elevation of the skin, and as such exhibits the characteristic tissue layers of skin. There are, however, certain very definite modifications, as will be seen.

1. *Epidermis.* The epidermis of the comb may be divided into three distinct layers. According to the terminology of Jeffries ('83), they are, proceeding from the deeper cells to the superficial, 1) mucous, 2) transitional, and, 3) flat horny cells (fig. 6).

The mucous layer (fig. 6, *M*), the deepest layer of the epidermis, consists of large, cylindrical cells, arranged vertically as a pallisade layer, and connected with the underlying dermis by fine cell processes.

Peripheral to the mucous layer lie several layers of transitional cells (*T*). The layer next to the mucous cells is distinguished from them by the fact that the cells are round in outline and smaller in size. With each more superficial layer, the cells become gradually less rich in protoplasm and more and more flattened. The most flattened of these cells form the flat horny cells (*H*), constituting the stratum corneum. At the periphery this process of cornification becomes so exaggerated that the horny cells lose their identity as cells and appear as layers of horny material.

2. *Dermis*. The dermis consists of connective tissue abundantly supplied with blood and lymph vessels. Within the dermis also is the nerve supply of the comb. Three layers may readily be distinguished in the dermis. Perhaps the best way to designate them is simply according to their position, as does Kostarew ('67), calling them, 1) the central, 2) the intermediate layer, and, 3) the peripheral layer (fig. 5).

At the tip of a point (pl. 1, *♂ 7mos.P*) in the mature male comb, the central region or core (fig. 5, *C*) constitutes one-fourth, the intermediate layer (*I*) a little more than one-fourth, and the peripheral layer (*P*) nearly one-half the total thickness of the comb.

The core proper is about the same thickness from point to base, but in the more basal regions large deposits of fat usually appear between the connective-tissue bundles, and the layer is consequently much enlarged. The intermediate layer in the basal region is far thicker than in the points; here it occupies fully two-thirds of the entire thickness of the comb. The peripheral layer is about the same thickness at the base and in the points, but it appears thinner at the base, due to the fact that there are fewer papillae (fig. 6, *pa*) in this region than in the points.

The most conspicuous element in the *central* region (fig. 5, C) is the longitudinally extended core of stout bundles of collagenic connective-tissue fibers, connected directly with the periosteum of the skull and extending in vertical and more or less parallel arrangement throughout the comb, even up into the points. Some of these fibers give off processes laterally, which are united with the connective-tissue fibers of the intermediate layer. In healthy birds a large quantity of fat is deposited in the basal part of the central region or core.

The central region is further differentiated from the other regions of the dermis by the presence of large thick-walled arteries and thin-walled veins, some of them as much as 0.5 mm. in diameter, which come in from the adjacent skin and extend vertically among the central connective-tissue bundles. These vessels enter the comb from the skin in the region of the orbit, branching as they go. Usually two or three vessels arise from each side of the head; the number varies. Each gives off branches to the basal area of the comb before proceeding into a point. From among the tributaries of these vessels each point is supplied with at least one large artery and one large vein. In close association with the artery and vein complex of each point appears a large nerve trunk.

The connective-tissue framework of the *intermediate* layer (fig. 5, I) in the cock's comb is much looser and softer than that of the central core. It consists for the most part of reticulum, with scattered groups of collagenic fibers and some elastic fibers (fig. 7). The layer as a whole presents a disorganized appearance, resembling the mucous connective tissue which forms the main mass of the umbilical cord in mammals. The connective-tissue cells of this layer appear large and star-shaped, with long, fine processes. The large interspaces between the cells are filled with a clear gelatinous substance which exudes when the fresh comb is cut and which gives a mucoid reaction. Accordingly, Champy and Kritch ('26) call this the muco-elastic layer. The general disorganized appearance of this layer is apparently due to the forcing apart of cells and cell processes already established, by a

rapid infiltration of the mucoid matrix. Fat deposits never occur in this layer.

The blood vessels connecting the large vessels of the core of the comb with the capillary network at the periphery are small and relatively inconspicuous. These tributaries of the central core vessels (fig. 7, *tr*) pass directly through the intermediate layer, giving off very few branches to this region. At the junction of the intermediate and peripheral layers, however, they empty into large arterioles (fig. 7, *ar*). From these arterioles arises a system of anastomosing capillaries, smaller at first, but growing larger and larger toward the periphery.

This capillary network forms the most conspicuous part of the *peripheral* layer. The papillae (fig. 6, *pa*) of the peripheral layer, which are apparent externally in the rough, granulated appearance of the cock's comb, particularly the points, show these capillaries greatly exaggerated in number and size. The regions between the papillae are supplied with smaller capillaries, relatively much fewer in number (fig. 6).

The turgidity of the cock's comb as compared with the comb of the capon and the hen is due both directly and indirectly to the presence of large quantities of intercellular mucoid. Mucoid is a highly viscous substance, and when liberated from the cells it exerts a considerable pressure. It is presumably secreted by the fibroblasts of the connective tissue, and thus spreads apart the cells and fibers by its action (fig. 7). Hence, it seems reasonable that the presence of the mucoid in itself should be the chief factor contributing to the turgid condition of the comb. Indirectly, the mucoid acts by compressing the thin-walled veins of the intermediate layer, the thick-walled arteries remaining relatively unaffected, thus increasing the pressure within the arterioles and capillaries. The increase in peripheral pressure resulting from this condition produces dilation in the capillaries, especially in those at the periphery. If this dilation were under purely nervous control, we would expect the comb to grow pale and deep in color intermittently; instead, both its color and turgidity are

permanent as long as it is under the influence of the hormone.

In direct support of the above hypothesis, Champy and Kritch ('26) report that the veins of the intermediate layer are larger in capons and non-laying hens (where they are not compressed by mucoid) than in cocks and laying hens.

By the ingrowth and branching of the capillaries and their distention under the indirect influence of the hormone, the connective tissue of the peripheral layer is reduced as compared with that in the capon (compare figs. 6 and 8). Immediately beneath the epidermis where the capillaries are largest, the adjacent capillary walls are practically in contact (fig. 6, *pa*). Less superficially, however, as the capillaries become smaller and smaller, the intervascular connective tissue becomes more and more abundant. There is very little reticulum in this layer in proportion to the amount of collagenic tissue, in direct contrast to the condition in the intermediate layer. The more compact arrangement of the fibers in the peripheral layer shows that there is no appreciable amount of mucoid secreted there.

The capillaries of the peripheral layer have pushed out to the epidermis, and it is the haemoglobin from their blood, which, showing through the thin epidermis, gives the color to the comb. The fact that the color is due to haemoglobin alone is demonstrated by the lack of pigment in the cells of the comb other than blood cells, and further by the fact that a comb drained of its blood becomes almost colorless.

This elaborate network of enlarged capillaries is a curious phenomenon. The only other tissues in the animal body which approach this condition are tissues adapted for the respiratory function. Examples of such tissues are the capillaries of the gill filaments in water forms, those of the lungs in land animals, and those of the allantoic membrane in the chick embryo. In none of these is there so thick an epidermal coating between the vascular network and the respiratory medium as in the case of the comb. There is, however, no evidence for a respiratory function of the comb.

Since the comb is a secondary sex character, the function of the subepidermal capillaries in giving the comb its color is important. Why coloration should be accomplished in this species by blood pigment and not by pigments laid down in the specific comb tissues, as is the case with the head furnishings of other birds, e.g., the guinea-cock (Champy and Kritch, '26), is a mystery.

By injection Kostarew demonstrated that lymph vessels are restricted to the main comb, being totally absent from the points. Further, most of these vessels lie in the central core of the comb; there are a few in the more superficial part of the intermediate layer, and none in the peripheral layer. Our technique brings us no evidence on this point.

The comb is sensitive, but there is no evidence of nervous regulation of its color, turgidity, or movement. There is every reason to believe that there is little or no nervous control of the hormone reaction. But very little is known about the innervation of the comb.

B. Structure of the capon's comb

If castration is performed before the conditioning effect of the testis has been felt in the comb, the rate of growth of the comb is retarded until it corresponds to the rate of growth of the body as a whole (Pézard's 'isogonic' growth, p. 302).

If, however, castration is performed after the comb has reached the mature condition, it begins immediately to regress, i.e., it becomes reduced in size and pale in color. Pézard ('18) gives several series of figures on the regression rates of combs after postpuberal castration. He states that signs of regression appear on the day after castration is complete. During the first week the comb may regress as much as a centimeter in length. The second week of regression, and often the third, brings about as much decrease in comb length as did the first. By the fourth week, however, the rate of regression is always slower, and it gradually decreases week by week until finally no change is noted. Usually the period of regression lasts from five to eight weeks, but in a very large comb regression may be traced for twelve weeks.

Histologically, the principal cause for the failure of the comb of the prepuberal castrate to grow lies in the intermediate layer. As will be seen later (compare Developmental history, p. 301), the intermediate layer of the adult male comb begins to be differentiated by the infiltration of mucoid as early as six weeks after hatching. This infiltration continues steadily until at four months the comb has reached its mature aspect both externally and histologically, though it has not yet reached its maximum size.

If castration occurs before the mucoid secretion has begun, without the stimulus of the hormone, no such secretion takes place. Consequently the fibers of the intermediate layer remain as close together as those of the peripheral layer, and no compression is exerted upon the blood vessels of the intermediate layer. Thus blood is free to enter and to leave the comb with equal ease; correspondingly the capillaries of the peripheral layer remain small and few in number (fig. 8). Thus the comb of the prepuberal castrate remains small in size and pale in color.

If, however, castration takes place at some time after mucoid secretion has begun, not only does the secretion cease, but all the mucoid which has been previously secreted disappears. This reduces whatever pressure was being exerted upon the veins of the intermediate layer, and the blood leaves the capillaries for a time at a greater rate than that at which it comes in, until equilibrium with the vessels of the core is established. With this loss of blood from the peripheral capillaries they become contracted to a considerable degree.

Histologically, the capon's comb differs from that of the cock in the following striking points, proceeding centrally from the periphery (compare figs. 5 and 8). The papillae are decidedly angular in outline, instead of plump and full. The horny layer is much increased in thickness, corresponding to the dry, scaly external appearance of the capon's comb. The capillaries of the peripheral layer are much smaller than those of the cock. This becomes evident externally in the pale color of the comb. The connective tissue just beneath the

epidermis is quite as abundant as the capillaries there, and more abundant in the less superficial regions.

The most outstanding difference in the capon and cock combs, however, lies in the intermediate layer. Here the connective-tissue cells and cell processes lie much closer together in the capon than in the cock, due to the cessation of mucoid secretion, and as a result the whole layer appears more compact, being distinguished from the peripheral layer only by the capillaries there. The intermediate layer now constitutes only two-fifths the total thickness of the comb, as compared to two-thirds in the case of the cock, and if the central core contains an unusual amount of fat, which it often does in the inactive capon, a smaller proportion still.

C. The response of the capon's comb to the male hormone

In order to study the effect of injection of the male hormone upon the capon comb tissue, capons prepuberally castrated some months previously by Doctor Domm were injected daily over periods of three to six weeks. The hormone preparation used was extracted from bull testes, according to the method outlined in the paper of Moore, Gallagher, and Koch ('29). It was prepared and standardized by members of the Department of Physiological Chemistry. As effective doses were used in all the experiments recorded here, the quantitative assays are not given.

1. The histological results of injection. Injection of the male hormone into a capon restores to a considerable extent the mature male condition in the comb, the completeness of the reaction being a function of the length of time during which the animal was injected. Under the conditioning effect of the hormone, mucoid is secreted in the intermediate layer. As this substance forms, the connective-tissue cells and cell processes are forced apart, and the layer as a whole increases considerably in thickness. As this intercellular substance increases in amount it exerts a pressure upon the thin-walled veins of the intermediate layer, and presumably the blood pressure in the peripheral layer is increased. It is certain,

at any rate, that the superficial capillaries become dilated simultaneously.

The redder color of the comb becomes apparent after two or three injections. As the amount of blood in the sub-epidermal vessels increases, the comb becomes deeper in color. Due to the presence of the mucoid and larger quantities of blood, both under pressure, the comb becomes turgid and erect, and after a long period can scarcely be recognized from that of the normal cock.

The heterogonic growth of the comb under injection is due largely to the increase in the thickness of the intermediate layer, brought about by the infiltration of mucoid, but the other tissues increase *pari passu* so as to maintain their due proportions. As this increase in size takes place, the horny layer of the epidermis sloughs off, and the comb becomes glossier than that of the capon. At the same time the papillae with their contained bundles of capillaries assume the granulated appearance characteristic of the cock.

2. *The mechanism of response.* That the presence of the male hormone conditions the infiltration of mucoid, and indirectly by means of the mucoid, the changes in the vascularity of the comb, we may be reasonably certain.

The degree of compactness with which the connective-tissue fibers of the intermediate layer are arranged, as shown in azan preparations, serves as an indicator of the mucoid content of the tissue, but it is evident that such observations must be confirmed by more direct means of approach.

Hence stains more or less specific for mucoid were employed. Mucicarmin gave negative results. The mucoid of the comb may differ in composition from mucin, for which mucicarmin is supposed to be specific. Champy and Kritch ('26), however, report positive results with both mucicarmin and mucihematein. But they give the preference to a Bresiline technique which they outline in the paper referred to above.

In our work, toluidine blue (technique described above) has proved useful in determining the relative amounts of

mucoid in the various types of comb. As a metachromatic stain, toluidine blue is unstable and unreliable, the metachromatism often becoming reversed upon standing, as a microchemical test it is not specific nor quantitative; but in determining the distribution of the mucoid through the tissue it is of considerable value. When the metachromatic reaction goes through properly, the connective-tissue fibers of the intermediate layer stain bluish purple, while in between them appears a homogeneous pink material, the mucoid matrix. For reasons not yet well understood, this staining often becomes reversed, so that the fibers stain pink and the mucoid blue. At all events, the homogeneity of the mucoid distinguishes it, and its distribution is easily studied.

The male comb shows all or most of the intermediate layer filled with mucoid. The capon comb stands at the other extreme, showing none, or almost none, of this substance; when capons are injected with male hormone, it reappears in varying amounts, depending on the amount of hormone injected. Female combs show much less mucoid than male, but more than injected capons, unless the hormone has been injected for an unusually long time, as in the case of bird 269, whose autoplasmic comb graft and comb stump as well were studied (compare pl. 4).

The most direct, though not necessarily the most conclusive, line of evidence for the relation of the hormone to mucoid content comes from the determination of the relative amounts of mucoid which can be extracted from combs of the various types. The method of extraction employed was, with minor modifications, that used by Chittenden and Gies ('96) for extracting mucoid from various types of connective tissue, tendon in particular.

The fresh comb is cut into small pieces and soaked in 10 per cent NaCl solution for an hour and then in distilled water for an hour. It is then dried on filter-paper and weighed, after which it is soaked in half-saturated $\text{Ca}(\text{OH})_2$ solution (4 cc. per gram of tissue) for three or four days, the solution being changed daily. The extracted mucoid gives

the solution a decidedly viscous character. After the extraction seems complete, the tissue is washed in water overnight, and after drying, weighed. The difference in weight is considered to be due to the mucoid extracted.

The following table gives the amounts of mucoid extracted from the various types of comb tissue. (The mucoid was reprecipitated from the extract by means of hydrochloric acid, but not in sufficient quantities to make weighing of value.)

TYPE OF COMB	GRAMS OF DRY TISSUE	GRAMS OF DRY TISSUE AFTER EXTRACTION	GRAMS OF MUCOID	WEIGHT OF MUCOID WEIGHT OF COMB
Cock	49.1	23.7	25.4	.517
Capon	1.3	1.3	—	—
Capon injected three weeks	3.6	3.2	.4	.111
Capon after one-month regression	8.8	7.2	1.6	.182
Hen (laying)	3.6	3.1	.5	.139

From the table it is evident that the series arranged in the order of mucoid content in terms of percentage by weight is as follows: 1) cock, 2) capon whose comb has been regressing for one month after castration, 3) hen, 4) injected capon, 5) capon. Thus the evidence from the extraction experiments coincides with that from the sections.

At least the physical process by which the mucoid causes the changes in the comb is indicated by the data presented above, but how the formation of mucoid is conditioned by the hormone is not clear. That the hormone control is by means of the blood stream, and not over nervous paths, is proved by the fact that combs grafted to other parts of the body react in the same way to the stimulus of the hormone as does the comb in the normal position (pp. 311, 312).

There is no positive proof that the mucoid is secreted by the cells of the intermediate layer. Indeed, there is as yet no proof that the intercellular substance of any connective tissue is secreted by the cells of the tissue. Neither increased size nor the presence of granules or globules can be used to

indicate secretory activity on the part of these cells. Perhaps studies on the mitochondria and the Golgi apparatus will soon give some evidence on this point, but as yet we have no criterion for determining whether a connective-tissue cell is secreting or not. Hence, we do not know that the fibroblasts of the intermediate layer secrete the mucoid, but it seems very probable that they do. It is hard to imagine any other source for the mucoid.

From the various lines of evidence here presented we feel justified in maintaining that, while the chemical reactions involved are not yet understood, the male hormone produces its effect upon the comb primarily by stimulating the cells of the intermediate layer to secrete mucoid. The data do not, however, exclude the idea of a direct effect of the hormone upon the capillaries or the growth of other tissues.

D. Structure of the mature hen's comb (figs. 10 and 11)

The female comb matures much later than does that of the male, and at maturity it differs quantitatively rather than qualitatively from the male.

At the laying periods the hen's comb enlarges and 'lops' considerably to one side or the other. Champy and Kritch ('26) have demonstrated that lopping is caused by an asymmetrical lateral increase in the number of connective-tissue fibers. These fibers may appear on either side of the comb. They are especially numerous halfway up the comb from the base. This mechanism brings about a feeble flexion when the comb is small, but when at laying the comb becomes large and heavy with blood and mucoid, the flexion is accentuated greatly (pl. 1, ♀ 5mo.). The mucoid in the comb of the laying hen is not sufficient in quantity to cause a turgor approaching that of the cock; if the hen's comb were as turgid as that of the cock, we would expect that this turgidity would counteract at least some of the lopping tendency.

When the hen is laying, its comb presents a histological picture strikingly different from that of a non-laying hen and much more like the cock's comb. However, even the laying

hen shows several characteristics which distinguish it from that of the cock. The connective tissue of the peripheral layer of the dermis is more compactly arranged in the hen (compare figs. 6 and 11); the capillaries are smaller and less numerous, and the arterioles which lie at the junction of the peripheral with the intermediate layer are less conspicuous. The connective-tissue cells and fibers of the intermediate layer lie closer together, due to the secretion of less intercellular mucoid; correlated with this fact, it is evident that the intermediate layer comprises a smaller proportion of the total thickness of the comb in the laying hen than in the mature cock. The core contains large fatty deposits, as does the core in combs of all birds in good physiological condition. Just after laying, however, many of the fat spaces appear collapsed, due probably to the fact that large quantities of fat have been utilized in the formation of yolk.

The reduction in the size of the comb of the non-laying hen—a fact which has been generally observed—is due primarily to a reduction in the thickness of the intermediate layer of the dermis. Evidently almost no mucoid is secreted in the periods between laying, for the connective tissue of this layer then appears so compactly arranged that it is distinguished from the peripheral layer only by the capillaries there (figs. 10 and 11). The capillaries are probably smaller between laying periods, but the difference is not striking. From a thickness in the laying hen almost twice as great as that of the core, at the base of the comb, the intermediate layer, in the non-laying hen, shrinks until it is nowhere as thick as the core. The horny layer of the epidermis becomes much thicker in the non-laying hen. In short, the comb of the non-laying female can scarcely be distinguished histologically from the comb of the capon.

The fat content of the core seems to be considerably increased in the periods between laying, if not absolutely, certainly proportionally to the total weight of the comb tissue. Smith ('11) states that this increase in fat content takes place just before the onset of laying, but large fat deposits

appeared in the combs of all non-laying hens studied, even in those sectioned before maturity. Further observations on this point will be presented in connection with the developmental history of the comb.

E. The mechanism of response to the female hormone

From the evidence which has been presented it seems clear that the differentiation of the intermediate layer may be taken as a criterion for the presence of the male hormone. There are several reasons for the conclusion that this differentiation may also be used as a criterion for the presence of the female hormone.

1. From the study of the histological development of the comb after hatching, which will be presented below, it appears that in the male the intermediate layer begins to differentiate six weeks after hatching, before the core is completely differentiated. In the female the core is well differentiated at two months, when there is not the least sign of differentiation in the intermediate layer, and it is seven months before differentiation of the intermediate layer is complete. These observations are well correlated with the fact that the testis develops gradually from hatching on, while the ovary remains dormant until about five months (tables 1 and 2). We realize that with our present knowledge of the subject, gonad size can scarcely be considered as a criterion of secretory function, still we feel sure that this correlation of comb size (pl. 1 and tables 1 and 2) and comb differentiation with gonad size is suggestive at least. The female hormone makes its effect upon the comb felt later in the life history of the bird, and its effect is never so intense as that of the male hormone, but the two effects are quite similar.

2. From the differences between the combs of laying and non-laying hens which have just been presented, it seems reasonably certain that the laying and non-laying combs stand in the same relation to each other as do the combs of cock and capon. When the ovary is in an active condition, the intermediate layer and capillaries show a type of differentiation,

which, since it is similar in kind if not in degree to that of the cock's comb, suggests the conditioning effect of the hormone. In the periods between laying the mucoid recedes and the capillaries contract; as a result the comb of the non-laying hen resembles that of the capon.

3. Injection of the female hormone causes slight growth in the combs of capon and hens (Juhn and Gustavson, '30). Here, again, we have important evidence that the female hormone produces a reaction which differs quantitatively rather

TABLE 1

Male

NO. OF BIRD	DATE OF DISSECTION	AGE IN DAYS	COMB LENGTH IN CENTI-METERS	COMB HEIGHT IN CENTI-METERS	L. TESTIS LENGTH IN MILLI-METERS	BODY WEIGHT IN GRAMS	$\sqrt{\text{BODY WT.}}$
2	8/10/28	18	1.3	.4	4	100	4.6
4		18	1.4	.4	4	100	4.6
6	8/11/28	19	1.4	.4	4	95	4.6
8		19	1.3	.3		86	4.4
10	5/30/28	30	2.3	.9	6	163	5.5
12	5/31/28	31	2.3	1.0	5	149	5.3
14	6/12/28	43	3.1	1.5	6	225	6.1
16	6/13/28	44	3.7	1.8	7	244	6.2
18	6/26/28	57	3.8	1.8	7	270	6.5
20	6/27/28	58	4.1	2.5	7	376	7.2
22	7/18/28	79	5.3	3.0	9	639	8.6
24	7/19/28	80	6.3	3.4	9	513	8.0
26	8/ 7/28	99	8.1	4.3		999	10.0
28	8/ 8/28	100	8.6	4.7	19	1124	10.4
30	9/ 5/28	128	7.6	4.0	9	1265	10.8
32	9/ 6/28	129	8.4	3.9	11	1514	11.5
34	10/ 8/28	161	9.6	4.8	19	1530	11.5
36	10/ 9/28	162	8.4	4.6	16	1671	11.9
38	11/ 7/28	191	9.3	4.6	16	1605	11.7
40	11/ 8/28	192	10.5	6.1	23	1683	12.0
42	12/ 6/28	220	10.5	6.1	23	1649	12.0
44	12/ 7/28	221	11.6	5.9	24	1523	11.5
46	1/10/29	255	12.6	8.3	23	1858	12.3
48	1/11/29	256	12.0	7.1	22	1740	12.0
50	2/ 5/29	281	13.3	7.4	29	2045	12.7
52		281	12.1	6.7		1838	12.3
54	3/ 4/29	308	12.1	6.1	27	1828	12.2
56	3/ 5/29	309	12.3	7.4		1544	11.6
58	4/ 1/29	336	14.1	8.1	30	1824	12.2
60	4/ 2/29	337	13.4	7.8	36	1698	11.9

TABLE 2

Female

NO. OF BIRD	DATE OF DISSECTION	AGE IN DAYS	COMB LENGTH IN CENTIMETERS	COMB HEIGHT IN CENTIMETERS	BODY WEIGHT IN GRAMS	♂ BODY WEIGHT	CONDITION OF OVARY
05	8/10/28	18	1.2	.3	75	4.2	Small, immature
07		18	1.1	.3	63	4.0	Small, immature
1	5/30/28	30	1.4	.5	128	5.0	Small, immature
3	5/31/28	31	1.4	.5	118	4.9	Small, immature
5	6/12/28	43	1.7	.4	215	6.0	Small, immature
7	6/13/28	44	2.1	.8	201	5.8	Small, immature
9	6/26/28	57	2.2	.6	285	6.6	Small, immature
11	6/27/28	58	2.3	.9	300	6.7	Small, immature
13	7/18/28	79	2.7	1.3	499	7.9	Small, immature
15	7/19/28	80	2.7	1.1	384	7.3	Small, immature
17	8/ 7/28	99	2.9	1.3	582	8.3	Small, immature
19	8/ 8/28	100	4.5	2.3	726	9.0	Small, immature
21	9/ 5/28	128	3.6	1.8	955	9.8	Small, immature
23	9/ 6/28	129	3.6	1.6	1028	10.1	Small, immature
25	10/ 8/28	161	4.4	1.9	1304	10.9	Follicles small
27	10/ 9/28	162	6.0	3.4	1180	10.6	Follicles very small
29	11/ 7/28	191	5.2	2.2	1238	10.7	Fairly large but immature
31	11/ 8/28	192	6.2	3.4	1235	10.7	Small follicles, further ad- vanced than 25 and 27
33	12/ 7/28	221	6.4	4.0	1552	11.6	Three very large follicles and two smaller ones
35	12/ 7/28	221	7.0	3.9	1553	11.6	Egg laid on day dissected. Five large follicles.
37	1/10/29	255	5.6	1.8	1271	10.8	Large follicle
39	1/11/29	256	6.9	3.9	1448	11.3	Small with two abnormal fol- licles
41	2/ 4/29	280	5.5	3.1	1815	12.2	Small with two medium-sized follicles
43	2/ 5/29	281	6.4	3.5	1472	11.4	Two rather small follicles
45	3/ 4/29	308	7.4	4.7	1589	11.7	Large follicles—largest ovary so far
47	3/ 5/29	309	7.0	3.6	1785	12.1	Egg in oviduct
49	4/ 1/29	336	6.7	4.5	1121	10.4	Four large follicles
51	4/ 2/29	337	6.3	3.8	1400	11.2	Three medium-sized follicles

than qualitatively from that of the male. Injection of male hormone extract into capons causes growth of the comb (McGee, Juhn, and Domm, '28). Injection of female hormone (extracted from the human placenta) causes the appearance of female feathers, and also "there are slight indications of an effect of the hormone upon the comb similar to those in the laying hen" (Juhn and Gustavson). One of the capons injected with female hormone showed an increase in comb length of 0.8 cm. during the period of injection. A comparable quantity of male hormone would have yielded considerably more growth, but from this it is evident that the female hormone produces some comb growth.

Thus as far as we have been able to judge from the techniques employed, the effects of the male and female hormones upon the comb differ in degree, but not in kind. The male hormone causes the stronger reaction, but each effects differentiation in the intermediate layer. The male and female hormones differ qualitatively with respect to the feather reaction (Juhn and Gustavson, '30) and presumably in other respects, but it is quite conceivable that substances which differ qualitatively from each other may produce reactions which differ only quantitatively. For example, two simple oxidating agents may be quite different in composition and yet both produce the same type of reaction; in one case, however, the reaction may go further than in the other. Hence, we conclude that the identity of action on the comb, so far as it exists, is no argument for chemical identity of male and female hormones.

THE COMB AS AN ORGAN

A. Origin and topography

The primordium of the comb first appears between the sixth and seventh days of incubation. It arises as do feather primordia and the primordia of the scales on the legs (Kerbert, '77), as an elevation of the ectoderm, caused by the rapid proliferation of underlying mesenchyme cells.

As early as eight days, feather germs (fig. 3, 10 *f*) begin to appear over the entire head with the exception of the beak (fig. 3, 10 *b*), the region about the eye, the region about the ear, and a narrow central region extending longitudinally from the beak posteriorly; the last is the comb area (fig. 3, 10 *a*). This comb area, so defined, becomes quite distinct at nine days. The comb becomes clearly differentiated from the beak by the tenth day. The line of demarcation between the comb and the beak is not straight, but notched, beak material filling in the notch (fig. 3, 12 *d*). This notch boundary between beak and comb remains throughout the life of the bird. The nostril slit (fig. 3, 2 *wks.n*) marks the ventral boundary of the comb, the anteroventral limit of the comb area.

The median comb elevation (fig. 3, 10 *e*), from which the comb itself arises, extends anteriorly as far as the beak, and until twelve days of incubation occupies longitudinally only the anterior half of the comb area and transversely no more than one-third of this area. As the embryo becomes more advanced, the comb, growing faster than the head, covers gradually more and more of the comb area, but never, even at maturity, does the comb occupy the entire area. There is always a region free from feathers and presenting the appearance of comb material, lying just posterior to the comb and on each side of it.

B. External structure of the adult comb

The single comb of the adult Brown Leghorn consists of three divisions, which may be described as follows: 1) An anterior serrated or unserrated region (fig. 3, 37 *mo.a*) of varying length, measured by the distance from the place where the comb joins the beak to the base of the first 'point' (Standard of Perfection of the American Poultry Association); 2) a posterior 'blade' (*A.P.A.*) with a varying number and arrangement of serrations and point-like structures, usually separated from the most posterior point by a larger space than are any two successive points separated from each other; 3) between 1 and 2 a series of points, varying in num-

ber, relative height and width, in the angle made with the base of the comb and in their relative distance apart. Points (fig. 3, *37mo.p*) are distinguished from serrations (*s*) by their greater size. Transitional stages between the two make diagnoses often quite arbitrary.

These points may be forked, serrated, or have irregular outgrowths upon them. Examination of over 150 of these combs showed that the number of points varied from three to eight, but that five is the class of greatest frequency, more than half the birds having combs with five points. This tendency to the five-pointed condition is undoubtedly due to selection by breeders, since the American Poultry Association specifies five points in its standard of perfection for the Brown Leghorn.

C. Embryonic development

The procedure for the study of the embryology of the comb consisted in photographing and sectioning the comb area of embryos from six days of incubation to hatching. The material was fixed in Bouin's fluid, and 10 μ sections stained in Mallory's triple stain and Heidenhain's azan.

The comb appears at the seventh day of incubation as a median longitudinal elevation. Shortly afterward, the primordia of the points and blade of the adult comb arise as small papillae, and by the ninth day are quite evident (fig. 3, 9). Sections show that they are merely local protuberances in which the mesoderm does not differ from that of the intervening regions of the comb elevation. In early stages the primordium of the blade cannot be distinguished from those of the points, except by the fact that it is most posterior in position, but by the fourteenth day the papilla which will form the blade is clearly larger than the others (fig. 3, 14).

In general, the development of the comb involves thickening and differentiation of the ectoderm to form the definitive epidermis, and multiplication and rearrangement of the mesenchyme cells, resulting in the formation of the three layers

of the dermis. This process is accompanied by the ingrowth and branching of blood vessels, causing the formation of capillary networks in the peripheral and central layers.

1. *Epidermis.* At seven days of incubation the epidermis appears in a simple, two-layered condition. Superficially, there is a layer of flat, polygonal cells, which Kerbert, in his report on the development of the scales, calls the epitrichial layer, and under this a single layer of round cells, which he calls the mucous layer. This mucous layer gives rise to the mucous layer of the definitive epidermis and also the transitional layer. Through the transitional layer it contributes to the formation of the horny layer, as does the epitrichial layer as well.

By the ninth day the mucous layer has begun to proliferate, and scattered between it and the epitrichial layer appear more round or oval cells, the beginning of the transitional layer. Outside the comb area the epidermis continues in this condition for some time, but within it and especially over the comb elevation proper, there is active cell division, resulting by the thirteenth day in four or five layers of cells. Thus the transitional layer of the definitive comb epidermis is formed.

Meanwhile the cells of the mucous layer, which heretofore have been round or oval, gradually appear elongated, and by the fourteenth day are definitely cylindrical in shape. By this time also the horny layer has begun to make its appearance, as a result of proliferation and flattening in the cells of the transitional layer. By eighteen days the epidermis has begun to assume the appearance of the epidermis in the adult comb. The adult condition is attained by further proliferation of cells from the mucous layer to form the transitional layer, and flattening of the cells of this transitional layer to form the flat, horny cells at the periphery.

2. *Dermis.* Sections of seven-day embryos show the mesenchyme of the comb elevation to be distinguished from the underlying mesenchyme by the fact that it is much more compact. At this stage this dense mesenchyme of the comb elevation is composed of round or oval cells. By the next day

some of the deeper cells of this region, instead of dividing rapidly and filling in every possible space as do the more superficial cells, become elongated horizontally until they are spindle-shaped (Stephenson, '15). This is the beginning of the central connective tissue characteristic of the adult comb. These spindle-shaped cells tend to arrange themselves in parallel layers. The tendency toward stratification is pronounced in the ten-day embryo.

With the increase in the height of the comb during succeeding days, caused by the rapid proliferation of the dense superficial layer of mesenchyme, we note an interesting rearrangement of cells. As the comb grows in height its two lateral areas tend to approach each other at the center, with the result that the comb becomes relatively thinner in proportion to its height. As this progresses the layers of fiber-like cells, which have been lying beneath the comb elevation proper, are pushed up in line with the upward growth, pressing together the cells ahead so that they form layers also.

With this rearrangement of the mesenchyme, the small blood vessels, which during the early stages are scattered through the basal region of the comb elevation, move upward into the comb itself, branching and rebranching as they go. At this stage the embryo is hatched.

D. Development of the comb from hatching to maturity

Throughout embryonic life, long after the gonad has become sex-differentiated, and for several weeks after hatching, male and female combs are indistinguishable, both grossly and microscopically.

The material for the study of the developmental history of the comb after hatching was taken from Doctor Juhn's 'normal series.' Every effort was made that the birds which form this series might be as near uniform material as possible. All the birds were hatched on the same date, and they were kept under identical conditions. At certain specified intervals (tables 1 and 2) four birds, two males and two females, were taken from the group, killed, and the various

organs measured, weighed, or photographed. As is apparent from examination of the tables, there are two birds for each determination and consequently for each point in our graph (comb or testis measurements and body weight).

This series furnished the photographs in the lower half of plate 1. From the data collected at the periodic autopsies, the body weights of the birds, their comb measurements, and in the case of the females, the descriptions of the gonads, were taken. Testis and ovary examinations were made from photographs of these organs (graphs and tables 1 and 2). The histological studies were made from sections of the same combs, one male and one female comb from the four birds killed at a given time, being used. After 221 days of age no combs were sectioned, for by this time one may be sure that, while comb growth may continue, still comb differentiation in both sexes is complete.

1. *Gross studies.* Pézard ('18) defines two types of growth of the comb. 'Isogonic' growth is growth which keeps pace with the growth of the whole organism, as represented by the body weight, and 'heterogonic' growth is growth which exceeds that of the organism as a whole, i.e., special or conditioned growth. The male comb shows heterogonic growth from a few weeks after hatching until maturity. In the case of castrated birds, after the preliminary reduction the comb becomes isogonic in its growth. Heterogonic growth may be restored by testis grafts or injection of the testis hormone.

After Pézard's method, the curves for the length of the comb and for the cube root of the body weight, reducing this value to linear terms, were plotted.

The curves (fig. 1) and photographs (fig. 3) for the male show that the comb, at least after the first month, grows at a rate much greater than the rate of growth of the animal as a whole—heterogonic growth. From puberty (the third month) to maturity there is a steady increase in the rate of growth of the male comb, and its divergence from the cube root of the body weight shows the conditioning effect of the testis hormone.

The female comb, however, exhibits isogonic growth until the fourth month (table 2, fig. 1 and fig. 3), when there is a very abrupt increase in the size of the comb, showing that heterogonic growth has begun. The photographs show an increase in the rate of female comb growth at that time even greater than that of the male at the same age. This sudden heterogonic growth is presumably conditioned by the female hormone. The series of comb measurements which appear in

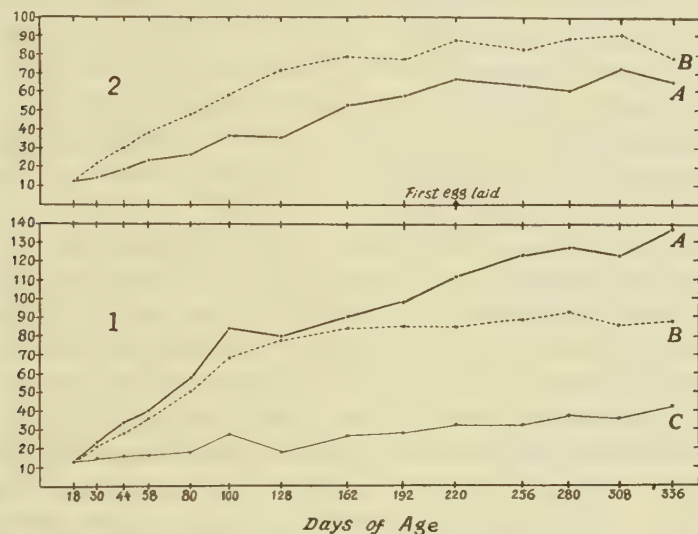


Fig. 1 1. Growth of male comb as compared with growth of testis and the body as a whole. 2. Growth of female comb as compared with growth of the body as a whole. Ordinate units represent: A) comb length, in millimeters; B) $10 \times$ body weight, in grams; C) left testis length, in millimeters.

table 2 place the onset of heterogonic growth at about five months of age. The ovaries of birds at this age show decided advances in development over those of all the younger birds examined. Since the sudden heterogonic growth of the female comb, which appears at the fourth or fifth month, is presumably conditioned by the female hormone, a correlation of comb size with ovary development would be expected. This correlation continues throughout the hens studied; in every case a large comb denotes a large, well-developed ovary (table 2).

Quite as striking as this correlation in the female is the correlation in the size of comb and testis in the cocks measured (table 1). The length of the left testis (measured from photographs about three-fourths natural size) was used as the criterion of the condition of the male gonad. Pézard states that the curves of growth for comb and testis are similar; but that there is no isochronism between the two, for the increase in the testis precedes that in the comb by several weeks. The data represented by table 1 and figure 1, however, point to a rather close correlation between the rate of growth of the comb and that of the testis. Nevertheless, we are inclined to doubt that any such correlation on the basis of measurements is valid, for it is quite common, especially in older birds, to find a very large comb accompanied by a very small testis, and vice versa.

Work on the freemartin (Lillie, '17) indicates that at least in cattle the testis hormone is secreted some time before that of the ovary. The fact that heterogonic growth of the comb arises much earlier in the cock than in the hen would indicate that the same situation may hold in the case of the fowl.

Unlike the cock's comb, after it has attained its full size the hen's comb exhibits striking and rapid fluctuations before and after laying (table 2). Smith ('11) points out that a marked increase takes place both in young and adult hens simultaneously with the periods of egg laying, and a decrease between these periods. The data represented by the curves also show this. There is a perceptible increase in comb size before laying, and a decided decrease after the first egg has been laid, with some indication of a second enlargement later. Histological studies confirm this opinion.

According to Smith, the increase in the female comb at laying is due to a fatty infiltration of the central connective-tissue core of the comb; the decrease, to the abstraction of this fat. On this point we cannot agree. No osmic-acid preparations, like those of Smith, were made, but, though the fat is dissolved by the technique used, the extent of fat deposits can easily be estimated by the distribution of the

empty fat cells. From these it appears that the amount of fat deposited in the core of the comb is a function of the general physiological condition of the bird in question, be it cock, hen, or capon. The increase in the hen's comb at laying is due rather to an increase in the thickness of the intermediate layer, due in turn to the infiltration of mucoid (see above). This is in harmony with the views of Champy and Kritch ('26).

2. *Histological studies—differentiation of the male and female combs from the common embryonic type.* From several days before hatching until a month thereafter there is a pause in comb differentiation in both males and females. Combs fixed eighteen days after hatching (compare fig. 4) may be taken as typical of this period. At this time there is yet no difference between sexes, either macroscopically or microscopically. The epidermis (*Ep*) has been differentiated for some time, but the dermis has made little progress toward differentiation. The peripheral and intermediate layers cannot yet be distinguished. Large bundles of collagenic connective tissue (*Co*) appear in the basal end of the central region, but that they will be later organized into core tissue is not yet apparent. A cushion of fat appears in the region beneath the comb, but none in the comb proper. The blood vessels at this stage are small and scattered.

At about a month after hatching, combs of male and female birds may be distinguished histologically and usually grossly. The female comb remains the same as before, while the male comb increases in size. Histologically, this increase in the male comb is correlated with a differentiation of the various layers of the dermis, with early increase in the thickness of the intermediate layer in particular. The difference in rate of differentiation of male and female combs at this period is correlated with the fact the testis grows rapidly in size, while the ovary remains small and immature for some time (tables 1 and 2).

Since at six weeks of age the difference between male and female combs is distinct, we shall, from this point on, consider the development of the two sexes separately.

a. Male. At six weeks the male comb appears to be differentiated as completely as the comb of the six-months-old female (compare fig. 3). The peripheral and intermediate layers of the dermis may be readily distinguished from each other. The capillary network of the peripheral layer is distinct. The connective-tissue fibers of the intermediate layer have separated somewhat, indicating that the infiltration of mucoid has begun; as a result of this reaction the intermediate layer has become much thicker than before. The collagenic fibers in the central part of the base of the comb have begun to arrange themselves longitudinally, and they, with the large blood vessels which appear in this region, give evidence of core differentiation. As yet no fat appears in the comb proper; it is restricted to the basal cushion described above.

At two months the comb shows little advance over the condition described above, except that the core has now reached its definitive condition. Large fat deposits appear within it. At this stage the core in the basal regions occupies almost one-third the thickness of the entire comb.

Eleven weeks of age finds the basal region of the comb completely differentiated. The capillaries of the peripheral layer are not so numerous as they will be later, but the intermediate layer is fully developed. Intermediate layer tissue comprises two-thirds the total thickness of the comb. At three months the capillary network has reached its definitive condition, and the arterioles between the peripheral and intermediate layers are conspicuous. The comb is mature in the basal regions, but differentiation of core structures in particular has not yet penetrated to the tips of the points. The advances in histological development at this stage are well correlated with the sudden increase in the size of comb and gonads (fig. 3, table 1, and fig. 1).

By four months the comb is mature throughout, from base to tip, and combs of cocks five, six, and seven months (compare fig. 5) of age show no further change except some increase in the size of the comb as a whole. In some combs

there is less fat in the central core, and the thickness of the core is reduced in consequence; this must be ascribed to the general physiological condition of the birds in question.

b. Female. Six weeks of age finds the female comb but little advanced over its condition at hatching. Peripheral and intermediate layers are still differentiated. The only evidence of core differentiation is the appearance of large blood vessels in the central region of the comb, and the presence of some fat tissue there, as well as beneath the comb proper.

By the time the bird has reached two months of age the capillary network begins to make its appearance. No further evidence of differentiation is apparent except in the central part of the basal region where stout bundles of collagenic connective tissue, longitudinally arranged, and large blood vessels indicate that the process of core formation is almost complete.

At eleven weeks the capillary network is further developed and core differentiation is complete. From this stage on fat deposits may appear throughout the core, particularly in the lower regions of the comb.

At three months the capillary network is completely differentiated, but the connective tissue of the intermediate layer is as yet indistinguishable from that of the peripheral layer.

Four months after hatching finds the capillary network still more conspicuous, and the arterioles at the base of the peripheral layer appear. The connective tissue of the intermediate layer appears somewhat more loosely arranged. Coincident with the establishment of the definitive blood supply, the hormone takes effect, and the infiltration of mucoid begins.

Up to this time the comb has remained small and the ovary has retained its immature condition, but by the fifth month the comb has suddenly increased considerably in size, and correlated with this increase the ovary shows small yolk follicles (figs. 1 and 3, table 2).

By six months the ovary is further developed, though still immature. Under the influence of the ovarian hormone, dif-

ferentiation of the intermediate layer has advanced further, but it is not yet complete, and the layer is still relatively thin, not as thick as the core at any point. Large fat deposits appear in the core.

At seven months (fig. 11) the ovary is mature—the bird studied laid an egg on the day it was killed—and differentiation of the intermediate layer is complete. The connective-tissue fibers appear disorganized, having been pushed apart by mucoid, and the layer reaches a thickness nearly twice as great as that of the core.

E. Comb transplantations

1. *Review of previous work.* The approach to the problem of the secondary sex characters by means of the transplantation of the comb or other tissues to other parts of the body has been attempted but little.

Caridroit ('25) reports the growth of two autoplasmic grafts of mature male comb points, and from this work concludes that outside its normal nervous connections, the tissue of the cock's comb remains under the conditioning influence of the testis hormone. Pézard ('26), using the same technique, found that after castration the grafted points regressed at the same rate as the points left in place on the comb.

Kozelka ('29), operating on white and brown Leghorns within the first three days after hatching, reports a series of thirty-nine successful transplantations of the entire comb, thirty-two autoplasmic and seven homoplasmic. The successful homografts were all between brothers and sisters.

This paper presents the results of 113 comb transplantations. As a by-product of these experiments come observations on regeneration in comb tissue. Work on the effect of castration and subsequent injection of the testis hormone in operated birds is also included.

Only autografts proved successful, but they were successful in over 80 per cent of the cases. The best results were obtained with combs grafted subcutaneously to the region beneath the wing.

2. *The unity of stump and graft.* The proportions of the comb, the number, size, and arrangement of its points and blade, as they appear in the embryo, are genetically determined and do not change with growth. Birds many months old may be identified by drawings made of their combs shortly after hatching.

An effort was made to identify the number, arrangement, and configuration of the points of the graft with those of the comb before its removal. The figure below gives drawings ($\times \frac{1}{2}$) of a number of the more successful grafted combs, 1) at the time of operation (two to four weeks of age) and, 2) four months afterward. Homologous points, where they may be identified, are numbered similarly (fig. 2).



Figure 2

Comparison of these two sets of drawings will show that in general they correspond. In some cases the points are homologized with difficulty, but these differences may be explained by the technique of the operation.

A piece of comb tissue never undergoes regeneration. This fact gave rise to the practice of 'dubbing' fighting cocks, i.e., cutting off comb and wattles to make them less vulnerable in battle.

Comb tissue, whether it be in its place upon the head or grafted to some other part of the body, is limited in its growth by that capacity which expresses itself in the normal growth of the comb. Graft and comb stump each develop into only that part of the comb which they would have formed if the comb had been left intact. Even if its parts are separated by space, the comb is a morphological whole.

There are several lines of evidence pointing to this conclusion:

1. When the stump has grown, it shows the irregularities of the original cutting.

2. The graft represents the part of the comb absent from the comb stump; points may be identified in the graft, but never in the stump (pl. 4).

3. Over four months after grafting, in one bird the graft measured at its base only 1 mm. less than the length of the stump along its upper margin. Corresponding measurements in a second bird three months after grafting showed only 2-mm. difference. The fact that there is a much greater discrepancy in the case of other birds may be explained by the fact that pieces of graft tissue often die and slough off before the graft has become established.

4. Histological examination of the comb stump reveals no difference between it and normal comb tissue. There is no evidence of any scar tissue which might have hindered growth.

3. *Histological studies of grafts.* Four weeks after grafting, when the birds were only six weeks of age, three (one male and two females) were killed and the grafts sectioned. At this stage all the graft tissue showed the histological

characteristics peculiar to the sex of the bird in question. In all cases, however, differentiation of the graft was not so far along as in combs of the same age, and the parts beneath the skin showed less differentiation than those protruding through the skin.

In order to study the histological situation in grafted comb tissue which had persisted until after maturity, sections were made of grafts nine months old or more. In general, comb grafts at maturity show the same differentiation of tissue layers as mature comb tissue in the normal position. The same sex differences occur, and the same effects follow castration and injection of the male hormone.

4. *Castration and injection in grafted birds.* Seven of the male birds bearing the largest grafts were castrated by Doctor Domm and injected daily with bull-testis hormone prepared in the Department of Physiological Chemistry. Bi-weekly measurements of both stumps and grafts were taken.

Bird 269 gave the most consistent and complete results, so we shall consider it as an example of this series of experiments. Plate 4 and the table below give the data for this bird.

Effects of castration and injection on bird 269

DATE		STUMP	WATTLES	GRAFT
3/10/29	Hatched			
3/29/29	Grafted			
11/18/29	Castration complete. Injections begun	58 × 17		36 × 32
12/13/29	Injections discontinued	58 × 17	71 × 55	36 × 32
1/17/30	Injections begun again	44 × 11	47 × 37	20 × 19
3/ 1/30	Injections discontinued. Bird killed shortly afterward	60 × 18	79 × 60	40 × 34

Injection was begun immediately after the second castration operation (the testes are removed separately). The hormone extract used had previously been found to be active by injection into a normal capon. As is apparent from the table, the graft and stump were maintained at the precastral level until (after twenty-six days) the injections ceased (com-

pare fig. 13). After a month with no injections, regression no longer took place (fig. 14). The stump had regressed to three-fourths its former length and two-thirds its former height, while the graft had been reduced to almost one-half its former size. The graft, being less rigidly attached, may be reduced further than the stump.

Injections for six weeks brought stump, wattles, and graft all back to their precastral condition (fig. 15 and table, p. 311). In fact, in each case the measurements are in excess of those made at the time of castration three and one-half months previously. This may be accounted for by the fact that in the absence of the conditioning effect of the testis, the comb grows, but only in proportion to the growth of the body as a whole—isogonic growth.

At the end of the experiment bird 269 was killed and its stump and graft sectioned. Both showed the typical mature male condition. There were large quantities of mucoid in the intermediate layer of both. Hence we may conclude that the increase in size of stump and graft after injection is due to the general reaction of comb tissue to the stimulus of the hormone.

A series of birds in which injections were begun at varying intervals after castration indicated that the ability of the graft to respond to the hormone becomes progressively less as the time after castration increases. This may be ascribed to a progressive inability of the blood vessels leading to the graft and within it to become redilated as the time of contraction in the absence of hormone stimulation increases. Evidently the blood vessels of grafts may become irrevocably contracted, while in the case of the comb in its normal position, this seldom happens. This situation may be understood when one considers the relatively thin stalk of tissue through which the blood vessels supplying the graft must travel, as compared with the mass of connective tissue which supports the comb in the normal position.

In general, both stump and graft regress upon castration and are restored by injection. Hence, it seems evident that

neither the upper nor the lower part of the comb is dependent upon the other for its response to the hormone.

SUMMARY AND CONCLUSIONS

1. The tissues of the comb may be homologized with the layers of the skin; the dermis, however, is decidedly modified.

2. The dermis of the mature male comb is divided into, 1) a central layer, or *core*, composed of stout bundles of connective tissue through which pass large blood vessels, 2) an *intermediate* layer of loose connective tissue whose fibers are widely spread apart by a matrix of mucoid, and, 3) a *peripheral* layer composed of compact connective tissue and an elaborate capillary network.

3. Castration prevents the mucoid from forming, or if (after puberty) it has already formed, castration causes its disappearance. Reduced blood pressure results, and the capillaries, especially those immediately beneath the epidermis, become smaller and possibly fewer in number. This correlates with the pale color and limp condition of the capon comb as compared with that of the cock.

4. Injection of the testis hormone restores the capon comb to the cock condition, microscopically as well as grossly.

5. The hormone produces its effect upon the comb primarily by stimulating the cells of the intermediate layer to secrete mucoid.

6. The laying hen's comb has an intermediate layer similar to that of the cock, but less highly developed and with less mucoid; in the non-laying hen's comb the mucoid disappears as in the capon. The action of the male and female hormones upon the comb is quantitatively different at least; the similarity, so far as it exists, is, however, no argument for their chemical identity.

7. The primordium of the comb first appears as an elevation of the ectoderm between the sixth and seventh days of incubation. Its development consists, in general, of thickening and differentiation of the ectoderm to form the definitive epidermis, and multiplication and rearrangement of the under-

lying mesenchyme cells, accompanied by ingrowth of blood vessels, resulting in the formation of the three layers of the dermis.

8. At first intermediate and peripheral layers cannot be distinguished, but as the influence of the hormone is felt, the intermediate layer becomes differentiated by the secretion of mucoid. The sexual differentiation of the comb in the male begins at four weeks; in the female, at two months. The comb may be said to be mature in the male at four months, but in the female not until seven months.

9. The size relations of the comb are genetically determined, and do not change with growth.

10. A piece of comb tissue never undergoes regeneration. When autoplasmic grafts are made, graft and stump each develop into only that part of the comb which they would have formed if the comb had been left intact.

11. Though it is usually retarded in its rate of differentiation, a graft at maturity shows the same sex differences and the same effects following castration and injection of the male hormone, both grossly and microscopically, as does mature comb tissue in the normal position.

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PLATE 1

EXPLANATION OF FIGURE

3 Series showing the development of the comb from seven days of incubation until seven months after hatching. (Age indicated on each photograph—days of incubation or age after hatching.) One day of incubation to one month after hatching ($\times 1$). Two months after hatching to seven months after hatching ($\times \frac{1}{7}$).

ABBREVIATIONS

<i>a</i> (in 10), comb area	<i>e</i> , comb elevation
<i>a</i> (in ♂7mos.), anterior region	<i>f</i> , feather germs
<i>b</i> (in 10), beak	<i>n</i> , nostril slit
<i>b</i> (in ♂7mos.), blade	<i>p</i> , point
<i>d</i> , notch of demarcation between beak and comb	<i>s</i> , serration



PLATE 2

EXPLANATION OF FIGURES

Figures 4, 5, 6, and 7 are photomicrographs of sections through male combs.

4 Male eighteen days after hatching ($\times 70$). $10\ \mu$; azan.

5 Male seven months old ($\times 15$). $20\ \mu$; azan. (Section through tip of a point.)

6 Mature male—peripheral region ($\times 100$). $10\ \mu$; azan. (Enlargement of papilla indicated in figure 5.)

7 Mature male—intermediate layer at point where tributary of core blood vessel enters arteriole ($\times 100$). $10\ \mu$; azan.

ABBREVIATIONS

<i>ar</i> , arteriole	<i>I</i> , intermediate layer
<i>C</i> , core	<i>M</i> , mucous layer
<i>co</i> , bundles of collagenic connective tissue to form core	<i>mu</i> , mucoid
<i>ct</i> , connective-tissue fiber	<i>P</i> , peripheral layer
<i>ep</i> , epidermis	<i>pa</i> , papilla
<i>F</i> , fat deposit	<i>T</i> , transitional layer
<i>H</i> , horny layer	<i>tr</i> , tributary of core vessel
	<i>cv</i> , blood vessel of core

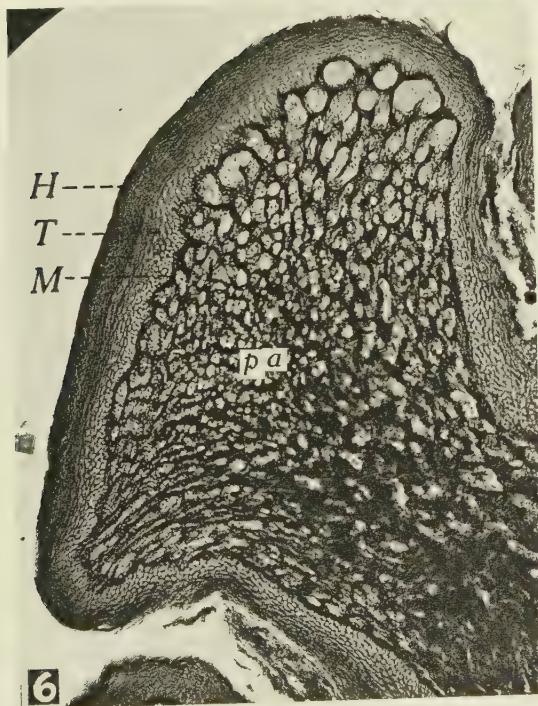


PLATE 3

EXPLANATION OF FIGURES

Figures 8, 9, 10, and 11 are photomicrographs of sections through capon and hen combs.

- 8 Capon prepuberally castrated ($\times 100$). $10\ \mu$; azan.
- 9 Capon after six weeks' injection ($\times 100$). $10\ \mu$; azan.
- 10 Non-laying hen ($\times 100$). $10\ \mu$; azan.
- 11 Laying hen ($\times 100$). $10\ \mu$; azan.

ABBREVIATIONS

<i>C</i> , core	<i>I</i> , intermediate layer
<i>cp</i> , epidermis	<i>P</i> , peripheral layer
<i>F</i> , fat deposit	<i>pa</i> , papilla
<i>H</i> , horny layer	

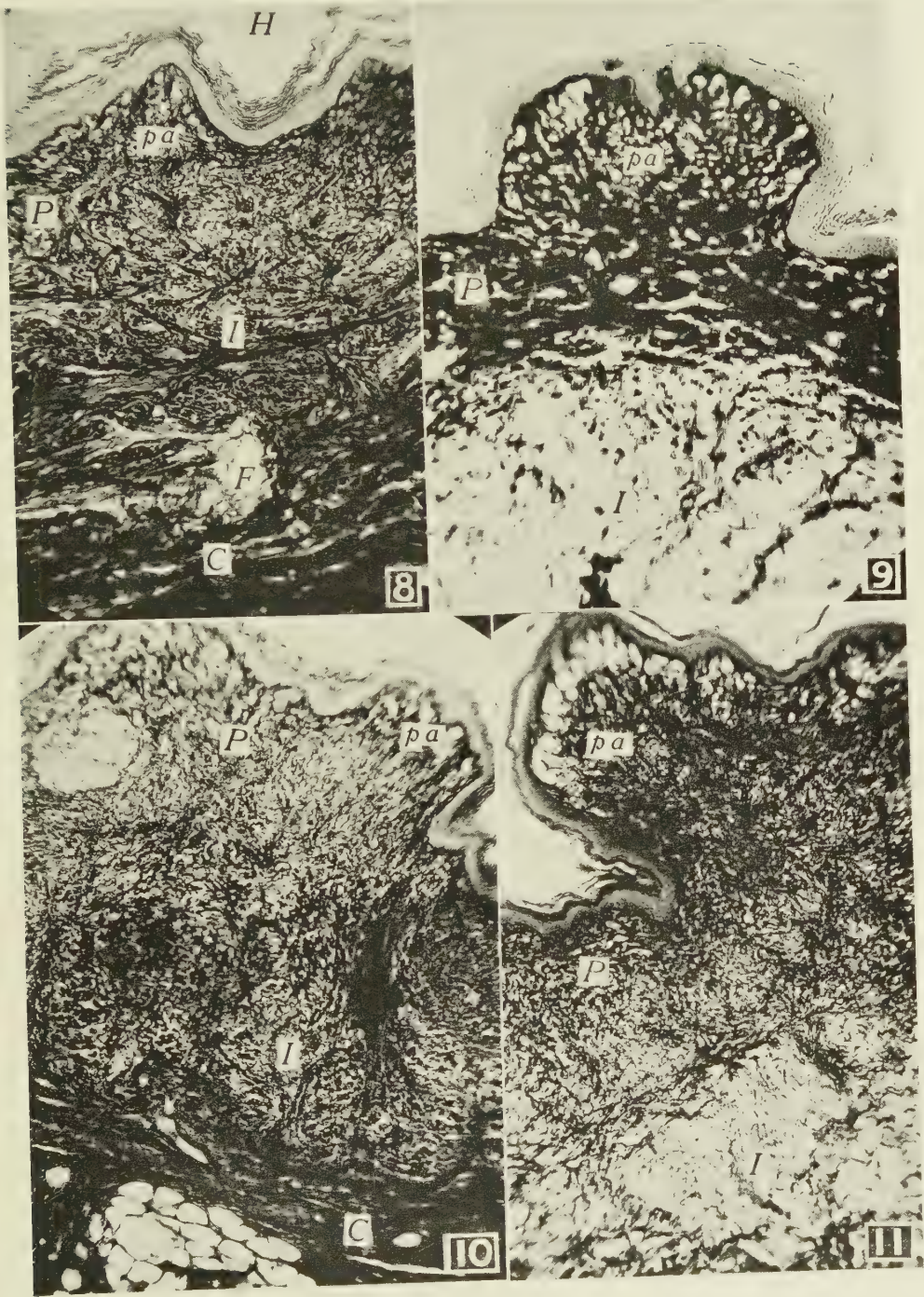


PLATE 4

EXPLANATION OF FIGURES

Figures 12, 13, 14, and 15 are photographs showing the effects of castration and injection upon the stump and graft of ♂ 269. Graft measurements are indicated. *z* = 1.

- 12 10/28/29; seven months after grafting; the day before castration.
- 13 12/7/29; castrate after three weeks of injection (injection begun immediately upon the completion of castration).
- 14 1 17/30; castrate showing regression after injections discontinued for a month.
- 15 3/5/30; castrate showing restoration after reinjection for six weeks.

ABBREVIATIONS

G, graft

S, stump



